PROCEEDINGS

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Celebrating Progress and Promise

protein, we have determined the complete sequences of the mouse (1) and rat BRCA1 coding regions. Surprisingly, the overall amino acid identity is only 58% between human and both rodent genes while the rat and mouse gene products are 81% identical. As expected, the RING finger motif near the amino terminus is essentially identical between the human and rodent BRCA1 proteins. Likewise, a pair of putative nuclear localization signals (rat codons 496–502 & 599–610) have been conserved perfectly. As with human and mouse, the rat protein carries a large net negative charge, including a region (codons 1301–1347) with 16 acidic residues and only one basic amino acid. A COOH terminal domain with helical character (rat codons 1582–1740) is well conserved (91 and 70% identity to mouse and human, respectively). Four of eight human missense mutation sites are clustered in this region. A putative tyrosine phosphorylation site, conserved among all three species, is located between the acidic and conserved carboxyl domains (rat codons 1509–1517). Finally, the bidirectional nature of the BRCA1 promoter region, first identified in humans, has been conserved in the mouse and rat genes. Progress in defining transcriptional control elements that regulate BRCA1 expression will be described.

#3519 Tuesday, April 23, 1996, 8:00–12:00, Poster Section 6

Suppression subtractive hybridization: A new method for cDNA subtraction of differentially expressed genes. Matz, M.V.[1], Diatchenko, L.[2], Chenchik, A.[2], Moqadam, F.[2], Huang, B.[2], Siebert, P.D.[2], Lau, Y.-F.C.[3], Campbell, A.[2], Lukianov, S.[1] *[1]Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia, [2]CLONTECH Laboratories, Inc., Palo Alto, CA, 94303, and [3]University of California, San Francisco, CA.*

A new and highly effective method, termed Suppression Subtractive Hybridization (SSH), has been developed for cDNA subtraction of differentially expressed genes, including the generation of subtracted cDNA libraries. It is based primarily on a recently described technique called suppression PCR and combines normalization and subtraction in a single procedure. The normalization step equalizes the abundance of cDNAs within the target population and the subtraction step excludes the common sequences between the target and driver populations. In a model system, the SSH technique enriched for rare sequences over 1,000 fold in one round of subtractive hybridization. We demonstrate its usefulness by generating a testis-specific cDNA library and by using the subtracted cDNA mixture as a hybridization probe to identify homologous sequences in a human Y chromosome cosmid library. The human DNA inserts in the isolated cosmids were further confirmed to be expressed in a testis-specific manner. These results suggest that the SSH technique is applicable to many molecular genetic and positional cloning studies for the identification of disease, developmental, cancer-related or other differentially expressed genes.

#3520 Wednesday, April 24, 1996, 1:00–5:00, Poster Section 11

Molecular genetic differences of differentiation in an Ara-C induced rhabdomyosarcoma cell line. Crouch, G. *Keesler Med. Ctr, Keesler AFB, MS 39534.*

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. These malignancies are thought to arise from myoblasts arrested along the normal pathway of differentiation. We have previously reported that RD, a human embryonal RMS cell line, displays a more differentiated phenotype following treatment with 0.5 micromolar (uM) cytarabine arabinoside (Ara-C). In fact, RD cells treated with Ara-C express increased amounts of actin and myosin by immunohistochemistry, form multinucleated giant cells by electron microscopy, have marked growth inhibition, and are less tumorigenic in nude mice. Ara-C is known to induce methylation changes in genomic DNA. This is one mechanism by which genetic expression is controlled. We performed restriction enzyme digests of DNA from Ara-C treated and untreated RD cells with methylation sensitive enzymes Hpa II and Msp I. Southern blot analysis using P(32)-dATP labeled alpha-actin promoter oligomer probe of these digested DNAs showed a difference in the methylation pattern of the RD genome. In order to investigate if these DNA changes were associated with altered gene expression, we analyzed the messenger RNA (mRNA) by differential display techniques. This method involved reverse transcription of the mRNAs obtained from Ara-C treated and untreated RD cells with oligo-dT primers, followed by polymerase chain reaction (PCR) in the presence of a second 10-mer. The amplified cDNA product was then visualized on a 6% polyacrylamide sequencing gel. The side-by-side comparison of mRNA from Ara-C treated and untreated RD cells identified differences in the expression of genes between the two groups of cells. The identification and further characterization of these differences in gene expression is in process.

#3521 Tuesday, April 23, 1996, 1:00–5:00, Poster Section 7

Tissue expression of the von Hippel-Lindau protein. M. Los, G.H. Blijham, G.H. Jansen, W.G. Kaelin Jr*, and R.R. Voest. *University Hospital Utrecht, The Netherlands, and *Dana Farber Cancer Institute, Boston, Ma.*

Von Hippel-Lindau (vHL) disease is an autosomal dominant inherited disorder, characterized by extensive vascularized tumors and cysts in specific organs. The vHL protein was recently shown to regulate transcription elongation by RNA polymerase II. To study the tissue expression of the vHL gene a panel of normal tissues was analyzed. Paraffin embedded tissue sections were incubated with a monoclonal antibody against vHL protein. Specific staining was observed in the cerebrum (the internal and external pyramidal layer), cerebellum (Purkinje and Golgi cells), and the myenteric and submucous plexus of the colon. Abundant staining was also observed in both endocrine and exocrine pancreas, adrenal tissue, renal tubuli, liver parenchyme, bile duct epithelium and colonic crypts. The cellular distribution of the vHL product was confined to the cytoplasm. Heart, lungs, smooth muscle cells and endothelium had no detectable expression. These findings demonstrate that vHL expression not only is present in the vHL target organs but also in organs not considered at risk for manifestations of the disease.

#3522 Tuesday, April 23, 1996, 1:00–5:00, Room 20

DD3: a new prostate specific marker, overexpressed in prostatic tumors. Bussemakers, M.J.G., Van Bokhoven, A., Ru, N.*, Isaacs, W.B.*. *Urology Research Laboratory, University Hospital Nijmegen, The Netherlands, *Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD*

In order to identify genes differentially expressed during prostate cancer development, we applied the technique of differential display analysis using mRNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients. We thus identified DD3, which on Northern blot detects two transcripts that are specifically expressed in human prostatic tumors. Nucleotide sequence analysis of DD3 did not reveal an open reading frame nor did we find homology with any known gene. Isolation of additional DD3-related cDNA and genomic clones allowed an initial characterization of the transcription unit of DD3 and revealed that alternative splicing may occur. Using the DD3-related genomic clones as probes, we were able to map DD3 to chromosome 9q21–22, a region which has been shown to be amplified in a number of prostatic tumors. RT-PCR analysis showed that DD3 expression is very prostate specific since no DD3-related product could be amplified in normal human artery, breast, bladder, colon, heart, kidney, liver, lung, muscle, pancreas, seminal vesicles, skin, spleen or testis. Also in the human prostate cancer cell lines LNCaP, Du145, PC3 and TSU, no DD3 transcripts could be detected. We are currently investigating whether we can use RT-PCR analysis of DD3 to detect prostate cancer cells in the peripheral blood of patients with metastatic disease. Furthermore, we will try to gain insight in the function of DD3 and its role in prostate cancer development.

#3523 Monday, April 22, 1996, 1:00–5:00, Poster Section 16

Induction and cellular location of aldose reductase and an aldose reductase-like gene in human primary liver cancers. Cao, D. L., Liu, X. F. L., Pan, S. T*, Chung, S. K. and Chung, S. M. S. *Institute of Molecular Biology and *Department of Surgery, the University of Hong Kong, 8 Sassoon Road, Hong Kong.*

We studied 9 human liver cancers and found that 2 of them expressed the aldose reductase (AR) gene, 2 expressed an aldose reductase-like gene (ARL-1), 1 expressed both AR and ARL-1. We cloned and sequenced ARL-1 cDNA and found that it encodes a protein of 316 amino acids with a 70.6% of its sequence identical to that of AR. The function of ARL-1 is not known yet, but its homology to AR and the conservation of key amino acids suggest that its activities may be similar to that of AR. It is not clear if AR and ARL-1 are required for the growth of the cancers that expressed these genes, but their differential expression in liver cancers may point to the different cellular origins or different stages of development of these cancers. This is being investigated by in situ hybridization to determine the cellular location of transcripts of these genes.

#3524 Sunday, April 21, 1996, 1:00–5:00, Poster Section 3

Isolation and characterization of the human NPM gene. Chan, P.K., Chan, F.Y., Leng, X.H., Morris, S.W.* and Liu, Q.R. *Baylor College of Medicine, Houston, TX 77030 and *St. Jude Children's Res. Hospital, Memphis, TN. 38101.*

NPM (nucleophosmin/B23) is a major nucleolar protein which is 20 times more abundant in tumor or proliferating cells than in normal resting cells. The putative function of NPM is ribosome assembly and transport. The t(5;2) chromosome translocation results from fusion of the 5' region of the NPM gene to the 3' region of an anaplastic lymphoma kinase (ALK) gene. To study the human NPM gene structure and expression, we isolated and characterized genomic clones from a human chromosome 5 library using a NPM cDNA probe. The total length of the NPM gene is over 25kb with 11 exons of sizes ranging from 58 to 358bp. The exon/intron junctions were identical to those of the rat NPM gene, suggesting NPM coding sequences are conserved. Intron #4, where t(5;2) translocation occurs, contains 2 Alu and T-rich sequences. The initiation site is located at 98bp upstream to the ATG site as determined by the primer extension method. The promoter region has a well defined TATA box (at −25) and a GC box (at −65). A group of cis-elements (UBF-1, ZRE-1, UCR core, E1A-P and EivF) related to viral gene expression were identified at −250 to −420. (Supported by NIH CA 42476.)

#3525 Monday, April 22, 1996, 1:00–5:00, Poster Section 14

Early overexpression of cyclin D1 in rat esophagus carcinogenesis. Youssef, E.M., Tukada, N., Morishima, Y., Hasuma, T., Otani, S., Higashino, M., Kinoshita, H., Fukushima, S. *1 Dept. of Pathol., 2 Dept. of Biochem. & 2 Dept. of Surg., Osaka City Univ. Med. Sch., Osaka 545, Japan.*

Overexpression of cyclin D1 has been reported from a number of investigations on human esophageal carcinomas. The aim of the present study was to assess the expression cyclin D1 in a group of esophageal tumors induced by nitrosomethylbenzylamine (NMBA) in rats. Samples for immunohistochemistry were obtained from thirty rats with esophageal tumors induced by s.c. injections, five times/week of 1.0 mg/kg NMBA for